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DUNSTON, JENNIFER ANN	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/091,177	COME ET AL.	
	Examiner	Art Unit	
	Jennifer Dunston	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *16 July 2007*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-37,39,40,42-63 and 65* is/are pending in the application.

4a) Of the above claim(s) *1-27,47,56-62 and 65* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *28-37,39,40,42-46,48-55 and 63* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *16 July 2007* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

This action is in response to the amendment, filed 7/16/2007, in which claims 38, 41, 64 and 66 were canceled, and claims 39-40, 42-45, 48, 50, 52 and 53 were amended. Currently, claims 1-37, 39-40, 42-63 and 65 are pending.

Applicant's arguments have been thoroughly reviewed, but are not persuasive for the reasons that follow. Any rejections and objections not reiterated in this action have been withdrawn. **This action is FINAL.**

Election/Restrictions

Applicant elected Group IV with traverse in the reply filed on 7/20/2005.

Claims 1-27, 47, 56-62 and 65 are withdrawn from consideration as being drawn to a non-elected invention. Applicant timely traversed the restriction (election) requirement in the replies filed on 7/20/2005 and 10/3/2005.

This application contains claims 1-27, 47, 56-62 and 65 drawn to an invention nonelected with traverse in the reply filed on 7/20/2005. A complete reply to the final rejection must include cancellation of nonelected claims or other appropriate action (37 CFR 1.144) See MPEP § 821.01.

Claims 28-37, 39-40, 42-46, 48-55 and 63 read on the elected invention and are currently under consideration.

Response to Arguments - Claim Objections

The objection of claim 42 has been withdrawn in view of Applicant's amendment to the claims in the reply filed 7/16/2007.

Response to Arguments - 35 USC § 112

The rejection of claim 45 under 35 U.S.C. 112, second paragraph, has been withdrawn in view of Applicant's amendment to the claims in the reply filed 7/16/2007.

Response to Arguments - 35 USC § 102

Applicant's arguments, see pages 23-24, filed 7/16/2007, with respect to the rejection of claims 28, 30-31, 33-34 and 52-53 under 35 U.S.C. 102(b) as being unpatentable over Keenan et al, as evidenced by Amara et al and Bierer et al, have been fully considered and are persuasive. The previous rejection of claims 28, 30-31, 33-34 and 52-53 has been withdrawn.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various

claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 54-55 are rejected under 35 U.S.C. 103(a) as being unpatentable over Keenan et al (Bioorg. Med. Chem. Vol. 6, pages 1309-1335, 1998; see the entire reference) as evidenced by Amara et al (PNAS, USA, Vol. 94, pages 10618-10623, 1997; see the entire reference) in view of Mehta (WO 00/07018; see the entire reference). This rejection was made in the Office action mailed 11/17/2006 and is reiterated below.

Keenan et al teach the method to identify binding of a polypeptide sequence to a user-specified ligand, comprising the steps of (i) providing a hybrid ligand such as dimerized FK1012 linked by polyehtylene linkers, (ii) introducing the hybrid ligand into a population of cells containing a SEAP reporter gene operably linked to ZFHD1 binding sequences, a first chimeric gene encoding a fusion protein containing three FKBP binding domains and a DNA binding domain from ZFHD1, and a second chimeric gene encoding a fusion polypeptide containing three FKBP binding domains and a transcription activation domain from the NF-κB p65 subunit, and (iii) allowing the hybrid ligand to bind the FKBP binding domains to induce dimerization such that transcription of the SEAP reporter gene is increased, and (iv) identifying positive ligand binding cells by activation of SEAP, and (v) identifying the nucleic acid sequence of the second chimeric gene (e.g. page 1334, Assay for inducible transcriptional activation; Figure 3; Table 1). Keenan et al teach that the method used to assay for inducible transcriptional

activation was performed as previously described by Amara et al (e.g. page 10620, right column, 1st full paragraph; paragraph bridging pages 10618-10619; Figure 4). Keenan et al teach the use of hybrid ligands, wherein R1 and R2 are FK1012 and Y is of the formula $(CH2\text{-}O\text{-}CH2)_n$, where n=2, 3, 4 or 5, (e.g. Table 1, compounds AP1427, AP1592, AP1511, and AP1578). The hybrid ligands taught by Keenan et al bind to FKBP with a dissociation constant (K_D) of less than 1 μM. Keenan et al teach that the affinity of structure 2d is threefold better than the original model monomer 2a, and the prior art teaches that the dissociation constant of FK506 to FKBP is in the nanomolar range (e.g. Bierer et al, page 9231, paragraph bridging columns). Bierer et al is used here only to demonstrate that the dissociation constants of the ligands taught by Keenan are less than 1 μM. Keenan et al teach the addition of the hybrid ligand to cells in the absence of the fusion proteins (e.g. Table 1, Apoptosis; page 1334, Assay for inducible Fas activation in cell lines). Keenan et al teach the measurement of SEAP activity in mock transfected cells to identify background SEAP activity in the absence of the hybrid ligand (e.g. page 1334, Assay for inducible transcriptional activation).

Keenan et al do not teach the use of a microtiter plate to confirm that the transcription of the reporter gene is dependent on the presence of the hybrid ligand.

Mehta et al teach the confirmation of the dependence of yeast three hybrid ligand interactions on the presence of both fusion proteins and the hybrid ligand by placing yeast cells in a well of a 96 well plate with hybrid ligand only or no hybrid molecule to serve as controls in the assay (e.g. Example 4). Further, Mehta et al teach the screening of libraries to identify numerous proteins that may interact with the hybrid ligand and teach the confirmation of the interactions using the 96-well microtiter assay (e.g. Example 1).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the yeast three hybrid assay of Keenan et al to include the use of the mcirotiter plate as taught by Mehta et al because both Keenan et al and Mehta et al teach it is within the ordinary skill in the art to use hybrid ligands in a yeast three hybrid assay. Further, it would have been obvious to conduct the assay on greater than 10 ligand-binding cell types because the assay can result in the identification of at least 10 ligand-binding cell types or can be repeated at least 10 times to identify 10 ligand-binding cell types.

One would have been motivated to make such a modification in order to receive the expected benefit of being able to use fewer reagents and to be able to perform more assays in less space by using the microtiter plate as taught by Mehta et al. Further, one would have been motivated to conduct the assay on at least 10 ligand-binding cell types in order to confirm each interaction identified in the screen. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 43-45 are rejected under 35 U.S.C. 103(a) as being unpatentable over Johnsson et al (US Patent No. 5,585,245, cited as reference P04 on the IDS filed 4/26/2003; see the entire reference) in view of Licitra et al (PNAS, USA, Vol. 93, pages 12817-12821, 1996, cited in a prior action; see the entire reference) and Bertozzi et al (J. Org. Chem., Vol. 56, pages 4326-4329, 1991, cited as reference CC on the IDS filed 7/20/2005; see the entire reference), as evidenced by Varshavsky et al (PNAS, USA, Vol. 93, pages 12142-12149, 1996, cited in a prior

action; see the entire reference). This is a new rejection, necessitated by the amendment of claim 43 in the reply filed 7/16/2007.

Johnsson et al teach a method of identifying the binding between a predetermined member of a specific-binding pair and a previously unidentified member of the specific-binding pair, comprising the steps of (i) providing a first DNA-based expression vector containing an expression cassette encoding a C-terminal subdomain of ubiquitin fused in frame to DNA encoding P1 and to a reporter moiety if P1 does not double as a reporter, (ii) providing a second DNA-based expression vector containing an expression cassette encoding randomly generated genomic or cDNA fragments fused to DNA (P2) encoding the N-terminal subdomain of ubiquitin (Nux), (iii) co-transforming a eukaryotic host cell with the first and second vectors such that the fusion proteins are produced, (iv) detecting cleavage of the fusion protein by the reconstituted ubiquitin moiety (e.g. column 9, lines 28-39; column 12, line 12 to column 13, line 20). Johnsson et al teach the method where the ubiquitin is reconstituted by the interaction of a ligand with P1 and P2 (e.g., Figure 5). Further, Johnsson et al teach that the C-terminal subdomain must bear an amino acid extension (i.e., to form Cub-Z) (e.g. column 6, lines 24-26). Thus, the first expressed fusion protein comprises segments P1, Cub-Z and RM, in an order where Cub-Z is closer to the N-terminus than RM (e.g. column 6, lines 24-26; column 12, line 12 to column 13, line 20; Figure 1D). Johnsson et al teach that the arrangement can also be reversed to have the randomly generated fragment fused to the C-terminal ubiquitin subdomain rather than the N-terminal subdomain (e.g. column 13, lines 15-20). Johnsson et al teach that the system may be used with transmembrane proteins (e.g. column 20, lines 34-45).

Varshavsky is cited only to show that N-end rule degradation operates in all organisms examined, from mammals to fungi and bacteria (e.g. Abstract; page 12147, left column, 1st full paragraph). Thus, the eukaryotic cells taught by Johnsson et al have an N-end rule degradation system.

Johnsson et al do not teach the step of providing a hybrid ligand represented by the general formula R1-Y-R2, wherein Y is a linker with the formula $(CH_2\text{-}O\text{-}CH_2)_n$, where n = 2-5.

Licitra et al teach a yeast three-hybrid assay, wherein a hybrid ligand of Dexamethasone and FK506 is used to screen a cDNA library for proteins capable of binding to FK506 (e.g. page 12818, Three-Hybrid Screen for FK506-Binding Proteins; Figures 1-3). A first hybrid protein comprising a DNA-binding domain and receptor for Dexamethasone (P1) was provided, and a second hybrid ligand comprising a potential receptor for FK506 (P2) and a transactivation domain was provided (e.g. Figures 2-4). Licitra et al teach the identification of positive interactions using the LacZ reporter gene (e.g. Figure 3). Licitra et al teach that the yeast three-hybrid assay has advantages over classical methods for identifying receptors for small ligand in that the system allows the direct isolation and identification of cDNAs encoding receptors and the system easily allows one to manipulate a large number of yeast colonies to study the structure-function relationship of ligand receptor interaction (e.g. page 12820, right column, last full paragraph). Further, Licitra et al suggest the use of other two- and three-hybrid systems that would allow the utility of the system to be expanded to other types of proteins such as membrane proteins (e.g. page 12820, right column, 1st full paragraph).

At page 4326, Bertozzi et al teach a linker of the following formula:

Bertozzi et al teach that polyethylene glycol derivatives are ideal for the purpose of linking two compounds to make bifunctional molecules for the study of enzymes and receptors, because they are inexpensive, water soluble, and available in a variety of lengths (e.g. page 4326, paragraph bridging column). Bertozzi et al teach that the heterobifunctional linker of the above structure contains a free amine that can be conjugated to biological molecules directly by an amide linkage (or via the corresponding isothiocyanate) and an azide that can be reduced to an amine for conjugation to other molecules (e.g. page 4326, right column). The free amine and azide of the structure of Bertozzi et al is an improvement of the prior art structures, because the Bertozzi structure is easier to link to biomolecules for the formation of bifunctional compounds (e.g. page 4326). Bertozzi et al exemplify a hybrid ligand comprising mannose linked to fluorescein with a $(CH_2\text{-}O\text{-}CH_2)_3$ linker (e.g. page 4327, left column).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the two-hybrid method of Johnsson et al to include the hybrid ligand, P1 and P2 portions taught by Licitra et al because Licitra suggest the use of other two- or three-hybrid systems to expand the utility of the assay comprising the hybrid ligand and Johnsson et al teaches a version of a three-hybrid method. Further, it would have been obvious to one of ordinary skill in the art at the time the invention was made to replace the linker of the hybrid ligand of Licitra et al with the linker taught by Bertozzi et al, because both references teach the use of a linker to link two moieties. Thus, it would have been obvious to one skilled in the art to

substitute one linker for another to achieve the predictable result of linking the two moieties for use in the screening assay.

One would have been motivated to make such a modification in order to receive the expected benefit of being able to identify membrane proteins capable of interacting with FK506 as suggested by Licitra and as taught by Johnsson et al. This modification would expand the utility of the hybrid ligand screening assay. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 28-34, 36, 46, 48-50, 52 and 53 are rejected under 35 U.S.C. 103(a) as being unpatentable over Liu et al (US Patent No. 5,928,868, cited in a prior action; see the entire reference) in view of Bertozzi et al (J. Org. Chem., Vol. 56, pages 4326-4329, 1991, cited as reference CC on the IDS filed 7/20/2005; see the entire reference). This rejection was made in the Office action mailed 11/17/2006 and is reiterated below.

Liu et al teach a screening assay for identifying a polypeptide sequence that binds to a user-specified ligand, comprising the steps of (i) providing a hybrid ligand having the general formula A-L-B (or R1-L-R2), where A is a first ligand and B is a user-specified ligand different from A, (ii) introducing the hybrid ligand into a sample containing a functional transcriptional and translational apparatus (for example, a whole cell) that includes vectors a encoding a hybrid protein including a transcription module and a target module for binding ligand A (P1) or for binding ligand B (P2) (target proteins #1 and #2), (iii) once the three hybrid complex comprising the hybrid ligand, first fusion protein and second fusion protein is formed, transcriptional

activation of a reporter gene occurs, and (iv) retrieving the plasmid encoding the fusion protein capable of binding to B and sequencing the plasmid (e.g. column 5. line 55 to column 8, line 46; column 11, lines 25-32; Figure 2). Liu et al teach that one of the vectors capable of binding the ligand contains a DNA binding domain and the other contains a transcription activation domain (e.g. column 7, lines 24-59; Figures 1-3). Liu et al teach that the nucleic acid sequence encoding the ligand B binding domain polypeptide is from random DNA sequences of a size that is capable of encoding a yet undetermined target protein, where the random sequences are derived from a genomic DNA library, cDNA library or synthetically generated library formed from eukaryotic cells, prokaryotic cells, viruses, or formed by an automated DNA synthesizer (e.g. paragraph bridging columns 7-8). With regard to the affinity of the hybrid ligand A to P1, Liu et al teach binding affinities including a Kd below 10^{-6}, 10^{-7}, 10^{-8} or 10^{-9} (e.g. column 8, lines 31-46). Liu et al teach that ligand A may be selected based upon a strong binding affinity for a target encoded by a fusion gene; the binding affinity must necessarily be measured if this determination is made (e.g. column 8, lines 31-46). Liu et al teach that A may form a covalent bond with P1 if a suicide inhibitor is used, for example beta-lactamase as P1 can covalently bind suicide inhibitors used as ligand A, including beta-lactam antibiotics (e.g. paragraph bridging columns 5-6). With regard to the reporter gene, Liu et al teach the use of LacZ, and GFP (e.g. column 8, lines 17-30; Figures 1-3). With regard to ligand B, Liu et al teach that the ligand may be selected from FK506, peptide libraries, nucleic acid libraries, polysaccharide libraries, and small organic molecules (e.g. column 6, lines 14-26). Liu et al teach the use of control experiments containing hybrid ligand only (without target proteins #1 and #2) to determine if the effects of the hybrid ligand are independent of trimeric complex formation (e.g. Example 5). Liu

et al teach the use of control experiments to confirm that reporter gene activation does not occur in the presence of the two target proteins in the absence of the hybrid ligand (e.g. column 11, lines 11-32). Liu et al teach that ligand A and ligand B may be covalently linked by any of the methods known in the art (e.g. column 6, lines 27-37).

Liu et al do not teach the linker between ligand A (or R1) and B (or R2) with the formula $(CH_2\text{-}O\text{-}CH_2)_n$, where n = 2-5.

At page 4326, Bertozzi et al teach a linker of the following formula:

Bertozzi et al teach that polyethylene glycol derivatives are ideal for the purpose of linking two compounds to make bifunctional molecules for the study of enzymes and receptors, because they are inexpensive, water soluble, and available in a variety of lengths (e.g. page 4326, paragraph bridging column). Bertozzi et al teach that the heterobifunctional linker of the above structure contains a free amine that can be conjugated to biological molecules directly by an amide linkage (or via the corresponding isothiocyanate) and an azide that can be reduced to an amine for conjugation to other molecules (e.g. page 4326, right column). The free amine and azide of the structure of Bertozzi et al is an improvement of the prior art structures, because the Bertozzi structure is easier to link to biomolecules for the formation of bifunctional compounds (e.g. page 4326). Bertozzi et al exemplify a hybrid ligand comprising mannose linked to fluoescein with a $(CH_2\text{-}O\text{-}CH_2)_3$ linker (e.g. page 4327, left column).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the hybrid ligand of the three-hybrid method of Liu et al to include the

linker comprising a free amine and azide of Bertozzi et al because Liu et al teach it is within the ordinary skill in the art to use any method known in the art to link ligands A and B to form a hybrid molecule and Bertozzi et al teach the use of the linker to form a hybrid ligand.

One would have been motivated to make such a modification in order to receive the expected benefit of easily linking ligands A and B as taught by Bertozzi et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 28-36, 46, 48-50, 52 and 53 are rejected under 35 U.S.C. 103(a) as being unpatentable over Liu et al (US Patent No. 5,928,868, cited in a prior action; see the entire reference) in view of Bertozzi et al (J. Org. Chem., Vol. 56, pages 4326-4329, 1991, cited as reference CC on the IDS filed 7/20/2005; see the entire reference) and Lin et al (Journal of the American Chemical Society, Vol. 122, pages 4247-4248 and supporting pages S1-S12, published online 4/13/2000, cited in a prior action; see the entire reference). This rejection was made in the Office action mailed 11/17/2006 and is reiterated below.

The teachings of Liu are described above and applied as before.

Liu et al do not teach the linker between ligand A (or R1) and B (or R2) with the formula $(CH_2\text{-}O\text{-}CH_2)_n$, where n = 2-5. Liu et al do not teach the three-hybrid method where A (or R1) is methotrexate.

At page 4326, Bertozzi et al teach a linker of the following formula:

Bertozzi et al teach that polyethylene glycol derivatives are ideal for the purpose of linking two compounds to make bifunctional molecules for the study of enzymes and receptors, because they are inexpensive, water soluble, and available in a variety of lengths (e.g. page 4326, paragraph bridging column). Bertozzi et al teach that the heterobifunctional linker of the above structure contains a free amine that can be conjugated to biological molecules directly by an amide linkage (or via the corresponding isothiocyanate) and an azide that can be reduced to an amine for conjugation to other molecules (e.g. page 4326, right column). The free amine and azide of the structure of Bertozzi et al is an improvement of the prior art structures, because the Bertozzi structure is easier to link to biomolecules for the formation of bifunctional compounds (e.g. page 4326). Bertozzi et al exemplify a hybrid ligand comprising mannose linked to fluoescein with a $(CH_2\text{-}O\text{-}CH_2)_3$ linker (e.g. page 4327, left column).

Lin et al teach a method of identifying a polypeptide sequence that binds to a user-specified ligand, comprising the steps of (i) providing a hybrid ligand comprising methotrexate linked to dexamethasone through a linker region, (ii) introducing the hybrid ligand into yeast cells comprising a LacZ reporter gene operably linked to a LexA binding site, a first chimeric gene encoding a fusion polypeptide of LexA and DHFR, a second chimeric gene encoding a fusion protein of GR and B42, (iii) allowing the hybrid ligand to bind the first and second fusion proteins to result in an increase in the level of the transcription of the reporter gene, (iv) identifying a positive ligand binding cell by detecting blue colonies of yeast grown on X-gal containing plates, and (v) identifying the nucleic acid sequence of the second chimeric gene (e.g.

page 4248, left column; Figures 1 and 2; Scheme 1; page S6). Further, Lin et al teach the assay where one of the fusion proteins is deleted to detect the effect of the hybrid ligand independent of the formation of the trimeric complex of the two fusion proteins and the hybrid ligand (e.g. page 4248, left column, last paragraph). Moreover, Lin et al teach the assay in the absence of the hybrid ligand to confirm that the transcription of the reporter gene is dependent on the presence of the hybrid ligand and fusion proteins (e.g. Figure 2). Lin et al teach that methotrexate can be modified readily without disrupting receptor binding, is commercially available, and has a picomolar affinity for DHFR (e.g. page 4247, right column, 1st paragraph).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the hybrid ligand of the three-hybrid method of Liu et al to include the linker comprising a free amine and azide of Bertozzi et al because Liu et al teach it is within the ordinary skill in the art to use any method known in the art to link ligands A and B to form a hybrid molecule and Bertozzi et al teach the use of the linker to form a hybrid ligand. Further, it would have been obvious at the time the invention was made to modify the hybrid ligand of Liu et al to include methotrexate as A (or R1), because Liu et al teach that A can be varied and Lin et al teach the use of methotrexate in a three-hybrid assay.

One would have been motivated to make such a modification in order to receive the expected benefit of easily linking ligands A and B as taught by Bertozzi et al. Further, one would have been motivated to use methotrexate as R1, because Lin et al teach that methotrexate can be modified readily without disrupting receptor binding, is commercially available, and has a picomolar affinity for DHFR. Based upon the teachings of the cited references, the high skill of

one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 28-34, 36, 46 and 48-53 are rejected under 35 U.S.C. 103(a) as being unpatentable over Liu et al (US Patent No. 5,928,868, cited in a prior action; see the entire reference) in view of Bertozzi et al (J. Org. Chem., Vol. 56, pages 4326-4329, 1991, cited as reference CC on the IDS filed 7/20/2005; see the entire reference) and Karlsson et al (US Patent No. 6,143,574, cited in a prior action; see the entire reference). This rejection was made in the Office action mailed 7/16/2007 and is reiterated below.

The teachings of Liu are described above and applied as before.

Liu et al do not teach the linker between ligand A (or R1) and B (or R2) with the formula $(CH_2\text{-}O\text{-}CH_2)_n$, where n = 2-5. Liu et al do not teach the use of plasmon resonance to determine the binding affinity of A to a fusion protein.

At page 4326, Bertozzi et al teach a linker of the following formula:

Bertozzi et al teach that polyethylene glycol derivatives are ideal for the purpose of linking two compounds to make bifunctional molecules for the study of enzymes and receptors, because they are inexpensive, water soluble, and available in a variety of lengths (e.g. page 4326, paragraph bridging column). Bertozzi et al teach that the heterobifunctional linker of the above structure contains a free amine that can be conjugated to biological molecules directly by an amide linkage (or via the corresponding isothiocyanate) and an azide that can be reduced to an amine for

conjugation to other molecules (e.g. page 4326, right column). The free amine and azide of the structure of Bertozzi et al is an improvement of the prior art structures, because the Bertozzi structure is easier to link to biomolecules for the formation of bifunctional compounds (e.g. page 4326). Bertozzi et al exemplify a hybrid ligand comprising mannose linked to fluoescein with a $(CH_2\text{-}O\text{-}CH_2)_3$ linker (e.g. page 4327, left column).

Karlsson et al teach that the BIAcore instrument uses the phenomenon of surface plasmon resonance to study the binding of analytes to receptors immobilized on a sensor chip to allow the affinity and kinetic analysis of interactions between soluble analytes and their immobilized binding partners to be determined (e.g. column 1, lines 11-45). Karlsson et al teach that affinity and kinetic properties for the solution interaction between an analyte and a binding partner can be determined by the following steps: (i) mixing the analyte with an immobilized binding partner (e.g. column 2, lines 3-15; column 3, lines 17-20). Karlsson et al teach that the method provides the ability to not only determine the true affinity properties but also true kinetic properties for the solution interaction between an analyte and binding partner therefore to thereby among other things be permitted a wider choice of reaction partners than in solid phase interactions and avoid immobilization artifacts (e.g. column 1, lines 59-65).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the hybrid ligand of the three-hybrid method of Liu et al to include the linker comprising a free amine and azide of Bertozzi et al because Liu et al teach it is within the ordinary skill in the art to use any method known in the art to link ligands A and B to form a hybrid molecule and Bertozzi et al teach the use of the linker to form a hybrid ligand. Further, it would have been obvious at the time the invention was made to include the use of plasmon

resonance to determine the binding affinity of A to P1, because Liu et al teach it is within the skill of the art to select A and P1 based upon binding affinity and Karlsson et al teach a method of determining binding affinity using plasmon resonance.

One would have been motivated to make such a modification in order to receive the expected benefit of easily linking ligands A and B as taught by Bertozzi et al. Further, one would have been motivated to use the plasmon resonance method of Karlsson et al in order to receive the expected benefit of providing the ability to not only determine the true affinity properties but also true kinetic properties for the solution interaction between an analyte and binding partner therefore to thereby among other things be permitted a wider choice of reaction partners than in solid phase interactions and avoid immobilization artifacts. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 28-34, 36, 46, 48-50, 52, 53 and 63 are rejected under 35 U.S.C. 103(a) as being unpatentable over Liu et al (US Patent No. 5,928,868, cited in a prior action; see the entire reference) in view of Bertozzi et al (J. Org. Chem., Vol. 56, pages 4326-4329, 1991, cited as reference CC on the IDS filed 7/20/2005; see the entire reference) and Licitra et al (PNAS, USA, Vol. 93, pages 12817-12821, 1996, cited in a prior action; see the entire reference). This rejection was made in the Office action mailed 7/16/2007 and is reiterated below.

The teachings of Liu are described above and applied as before.

Liu et al do not teach the linker between ligand A (or R1) and B (or R2) with the formula $(CH_2\text{-}O\text{-}CH_2)_n$, where n = 2-5. Liu et al do not teach the step of providing access to data, nucleic acids or peptides obtained from the identification of polypeptide binding to a hybrid ligand.

At page 4326, Bertozzi et al teach a linker of the following formula:

Bertozzi et al teach that polyethylene glycol derivatives are ideal for the purpose of linking two compounds to make bifunctional molecules for the study of enzymes and receptors, because they are inexpensive, water soluble, and available in a variety of lengths (e.g. page 4326, paragraph bridging column). Bertozzi et al teach that the heterobifunctional linker of the above structure contains a free amine that can be conjugated to biological molecules directly by an amide linkage (or via the corresponding isothiocyanate) and an azide that can be reduced to an amine for conjugation to other molecules (e.g. page 4326, right column). The free amine and azide of the structure of Bertozzi et al is an improvement of the prior art structures, because the Bertozzi structure is easier to link to biomolecules for the formation of bifunctional compounds (e.g. page 4326). Bertozzi et al exemplify a hybrid ligand comprising mannose linked to fluoescein with a $(CH_2\text{-}O\text{-}CH_2)_3$ linker (e.g. page 4327, left column).

Licitra et al teach a yeast three-hybrid assay, wherein a hybrid ligand of Dexamethasone and FK506 is used to screen a cDNA library for proteins capable of binding to FK506 (e.g. page 12818, Three-Hybrid Screen for FK506-Binding Proteins; Figures 1-3). Licitra et al teach the identification of positive interactions using the LacZ reporter gene and disclose the data in the publication (e.g. Figure 3). Licitra et al teach that the yeast three-hybrid assay has advantages

over classical methods for identifying receptors for small ligand in that the system allows the direct isolation and identification of cDNAs encoding receptors and the system easily allows one to manipulate a large number of yeast colonies to study the structure-function relationship of ligand receptor interaction (e.g. page 12820, right column, last full paragraph).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the hybrid ligand of the three-hybrid method of Liu et al to include the linker comprising a free amine and azide of Bertozzi et al because Liu et al teach it is within the ordinary skill in the art to use any method known in the art to link ligands A and B to form a hybrid molecule and Bertozzi et al teach the use of the linker to form a hybrid ligand. Further, it would have been obvious to provide the public access to the data through publication as taught by Licitra et al, because Liu et al teach a three-hybrid assay and Licitra teach a three-hybrid assay and provide the data obtained from the assay.

One would have been motivated to make such a modification in order to receive the expected benefit of easily linking ligands A and B as taught by Bertozzi et al. One would have been motivated to publish the data obtained from such an assay to be able to communicate the findings to peers in the form of a publication. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 28-34, 36-37, 39-40, 42, 46, 48-50, 52 and 53 are rejected under 35 U.S.C. 103(a) as being unpatentable over Liu et al (US Patent No. 5,928,868, cited in a prior action; see the entire reference) in view of Bertozzi et al (J. Org. Chem., Vol. 56, pages 4326-4329, 1991, cited

as reference CC on the IDS filed 7/20/2005; see the entire reference) and Zaharevitz et al (Cancer Research, Vol. 59, pages 2566-2569, cited in a prior action, 1999; see the entire reference). This rejection was made in the Office action mailed 7/16/2007 and is reiterated below.

The teachings of Liu et al are described above and applied as before.

Liu et al do not teach the linker between ligand A (or R1) and B (or R2) with the formula $(CH_2\text{-}O\text{-}CH_2)_n$, where n = 2-5. Liu et al do not teach the method where ligand B is a cyclin dependent kinase inhibitor of Table 2.

At page 4326, Bertozzi et al teach a linker of the following formula:

Bertozzi et al teach that polyethylene glycol derivatives are ideal for the purpose of linking two compounds to make bifunctional molecules for the study of enzymes and receptors, because they are inexpensive, water soluble, and available in a variety of lengths (e.g. page 4326, paragraph bridging column). Bertozzi et al teach that the heterobifunctional linker of the above structure contains a free amine that can be conjugated to biological molecules directly by an amide linkage (or via the corresponding isothiocyanate) and an azide that can be reduced to an amine for conjugation to other molecules (e.g. page 4326, right column). The free amine and azide of the structure of Bertozzi et al is an improvement of the prior art structures, because the Bertozzi structure is easier to link to biomolecules for the formation of bifunctional compounds (e.g. page 4326). Bertozzi et al exemplify a hybrid ligand comprising mannose linked to fluoescein with a $(CH_2\text{-}O\text{-}CH_2)_3$ linker (e.g. page 4327, left column).

Zaharevitz et al teach a small molecule cyclin dependent kinase inhibitor found on page 1 of instant Table 2 (e.g. Figures 1 and 4). Zaharevitz et al teach that these compounds are novel and are able to interact with a subset of CDKs (e.g. paragraph bridging pages 2568-2569). Further, Zaharevitz et al teach that the disclosed compounds are useful as a tool for exploring the structural bases and pharmacological significance of various kinase specificites.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the hybrid ligand of the three-hybrid method of Liu et al to include the linker comprising a free amine and azide of Bertozzi et al because Liu et al teach it is within the ordinary skill in the art to use any method known in the art to link ligands A and B to form a hybrid molecule and Bertozzi et al teach the use of the linker to form a hybrid ligand. Further, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the hybrid ligand of the three-hybrid method of Liu et al to include the CDK inhibitors disclosed by Zaharevitz et al as ligand B, because Liu et al teach that ligand B may be selected from a small molecule library and Zaharevitz et al teach that the kinase inhibitor is a small molecule.

One would have been motivated to make such a modification in order to receive the expected benefit of easily linking ligands A and B as taught by Bertozzi et al. Further, one would have been motivated to use the kinase inhibitors of Zaharevitz et al as ligand B to be able to screen for other kinases capable of binding the inhibitors to further characterize the kinase specificities of the inhibitors. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 28-34, 36, 46, 48-50, 52 and 53 are rejected under 35 U.S.C. 103(a) as being unpatentable over Liu et al (US Patent No. 5,928,868, cited in a prior action; see the entire reference) in view of Holt et al (WO 96/06097, cited as reference AD on the IDS filed 4/28/2003; see the entire reference). This rejection was made in the Office action mailed 7/16/2007 and is reiterated below.

The teachings of Liu are described above and applied as before.

Liu et al do not teach the linker between ligand A (or R1) and B (or R2) with the formula $(CH_2\text{-}O\text{-}CH_2)_n$, where n = 2-5.

Holt et al teach the formation of heterodimers of immunophilin ligand moieties (e.g. FK506 and derivatives thereof) (e.g. page 1; page 6, lines 12-17). Holt et al exemplify structures linked by polyethylene linkers of the formula $(CH_2\text{-}O\text{-}CH_2)_n$, where n = 1, 2, 3, 4 (e.g. pages 14-15). Holt et al teach the use of the homodimeric ligands comprising the abovementioned linker where n = 2 or 3 in an cell-based transfection assay where a trimeric complex comprising the homodimeric ligand, a fusion protein comprising three copies of FKBP12 fused to a Gal4 DNA binding domain, and a fusion protein comprising three copies of FKBP12 fused to a VP16 activation domain was detected by the production of the reporter product, secreted alkaline phosphatase (e.g. pages 48-49). Holt et al teach that multimerizers vary somewhat in their observed activity, depending upon the particular chimeric proteins and other components of the system and recommend that the practitioner select multimerizers based upon their performance in the particular system of interest (e.g. page 48 lines 26-30).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the hybrid ligand in the three-hybrid assay to include the $(CH_2\text{-}O\text{-}CH_2)_n$ linker of Holt et al because Liu et al teach it is within the ordinary skill in the art to use any linker known in the art and Holt et al teach linkers for making homodimeric or heterodimeric ligands capable of forming a trimeric complex in three hybrid assay.

One would have been motivated to make such a modification in order to receive the expected benefit of being able to vary the linker of the hybrid ligand to determine which ligand performs best in the three-hybrid system as taught by Holt et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Response to Arguments - 35 USC § 103

With respect to the rejection of claims 54-55 under 35 U.S.C. 103(a) as being unpatentable over Keenan et al, as evidenced by Amara et al, in view of Mehta, Applicant's arguments filed 7/16/2007 have been fully considered but they are not persuasive.

The response asserts that because claim 28 was not rejected under 35 U.S.C. 103(a), claims 54 and 55 cannot be obvious over Keenan et al in view of Mehta. This is not found persuasive, because claim 28 was rejected under 35 U.S.C. 102(b) and thus not included in the rejection made under 35 U.S.C. 103(a). While additional claims could have been included in this rejection, the absence of these claims in the rejection statement does not invalidate the rejection of claims 54 and 55.

The response asserts that Keenan is not interested in identifying any proteins that bind to R2, and the response asserts that Mehta would not remedy this deficiency even if there were additional motivation to combine Mehta. This is not found persuasive, because Mehta teach the screening of libraries to identify numerous proteins that may interact with a hybrid ligand (see the paragraph bridging pages 9-10 of the Office action mailed 11/17/2006; see also Mehta, Abstract, paragraph bridging pages 3-4). Thus, the combined teachings of Keenan et al and Mehta et al result in the claimed invention for claims 54 and 55. One would have been motivated to identify proteins capable of binding to a ligand, as taught by Mehta.

The response asserts that Keenan teach away from the claimed linkers because they perform poorly. This is not found persuasive, because use of the linkers results in some effect which would result in a predictable outcome in the claimed assay.

For these reasons, and the reasons made of record in the previous office actions, the rejection is maintained.

The rejection of claims 43-45 under 35 U.S.C. 103(a) as being unpatentable over Johnsson et al in view of Licitra et al, as evidenced by Varshavsky et al, has been withdrawn in view of Applicant's amendment to the claims in the reply filed 7/16/2007. Johnsson et al and Licictra et al do not teach the required polyethylene linker.

With respect to the rejections of record that rely on the combination of Liu in view of Bertozzi, Applicant's arguments filed 7/16/2007 have been fully considered but they are not persuasive.

The response asserts that there is no motivation to combine Liu with Bertozzi, since Bertozzi teaches away from using the PEG linker in the screening methods described in Liu.

The response essentially asserts that Bertozzi teaches away from the combination, because Bertozzi teaches that PEG linkers are water-soluble, and the linked compounds would have increased water solubility (hydrophilicity), which would be less likely to be membrane permeable. Further, the response asserts that one would have been motivated to look for hydrophobic moieties rather than hydrophilic moieties for application in the method of Liu et al so that the compounds would be capable of crossing the cell membrane. This is not found persuasive, because the method of Liu et al does not require the compounds to be capable of crossing the cell membrane. Liu et al teach that the hybrid ligand is introduced into the cell by traversing the membrane, or by electroporation or any permeation procedure that is known in the art (e.g., column 7, lines 3-15). Thus, the hybrid ligand is not required to be hydrophobic to be used in the method of Liu et al.

For these reasons, and the reasons made of record in the previous office actions, the rejection is maintained.

The rejection of claims 38 and 41 under 35 U.S.C. 103(a) as being unpatentable over Liu et al in view of Bertozzi et al and Zaharevitz et al is moot in view of Applicant's cancellation of the claims in the reply filed 7/16/2007.

The rejection of claims 38-40 and 42 under 35 U.S.C. 103(a) as being unpatentable over Liu et al in view of Zaharevitz et al is moot in view of Applicant's cancellation of claim 38 and amendment of claims 39-40 and 42 to depend from claim 37 in the reply filed 7/16/2007.

With respect to the rejection of claims 28-34, 36, 46, 48-50, 52, 53 under 35 U.S.C. 103(a) as being unpatentable over Liu et al in view of Holt et al, Applicant's arguments filed 7/16/2007 have been fully considered but they are not persuasive.

The response asserts that there is no teaching in Holt that would suggest that the PEG linkers would increase the cellular uptake of the hybrid ligands despite its hydrophilic structure. The response asserts that one would not have been motivated to select the subgenus of PEG linkers from the numerous disclosed linkers. Further, the response asserts that one would not have chosen a PEG linker-based construct when seeking to solve the technical problem of providing an improved membrane permeable heterodimeric hybrid ligand useful for *in vivo* application, such as the screening methods of Liu, due to the water-soluble nature of the linker. This is not found persuasive because the method of Liu et al does not require the compounds to be capable of crossing the cell membrane. Liu et al teach that the hybrid ligand is introduced into the cell by traversing the membrane, or by electroporation or any permeation procedure that is known in the art (e.g., column 7, lines 3-15). Thus, the compounds are not required to be membrane permeable for use in the assay of Liu et al. Furthermore, the compounds of Holt et al that contain the polyethylene linker were membrane permeable and found to have dimerizer activity within human 293 cells (e.g., pages 48-49). Both Liu et al and Holt et al teach the binding of a hybrid ligand to two chimeric proteins to activate gene expression in a cell, and thus it would have been obvious to one of skill in the art to substitute one linker for another in the hybrid ligand of the method in order to achieve the predictable result of dimerizing to protein ligands, regardless of whether the ligand is membrane permeable or delivered to the cell by another means known in the art, such as electroporation, to achieve the predictable result of providing a functional hybrid ligand for the assay of Liu et al.

For these reasons, and the reasons made of record in the previous office actions, the rejection is maintained.

Conclusion

No claims are allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Dunston whose telephone number is 571-272-2916. The examiner can normally be reached on M-F, 9 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach can be reached at 571-272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications

may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Jennifer Dunston, Ph.D.
Examiner
Art Unit 1636

/JD/

CELINE QIAN, PH.D.
PRIMARY EXAMINER